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Evaluation of *in vitro* antioxidant activity of various extracts of aerial parts of *Chomelia asiatica* (Linn)

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ABSTRACT

The study was designed to examine the invitro antioxidant activities of various extracts of aerial parts of Chomelia asiatica. The antioxidant activity was evaluated by DPPH (α,α-diphenyl-β-picrylhydrazyl) radical scavenging activity, Super Oxide Anion Scavenging Activity with reference standard Rutin, Quercetin respectively and estimate the amount of total phenol. The ethyl acetate extract of Chomelia asiatica was found to more effective in the DPPH radical scavenging activity. The IC_{50} of the ethyl acetate extract of Chomelia asiatica and Rutin were found to be 460µg/ml and 480µg/ml respectively. An IC_{50} value was found that ethyl acetate extract of Chomelia asiatica is more effective in scavenging superoxide radical than that of methanol and petroleum ether extract. But when compare to the all the three extracts with Quercetin (standard), the ethyl acetate extract of the Chomelia asiatica showed the similar result. In addition, the ethyl acetate extract of Chomelia asiatica was found to contain a noticeable amount of total phenols, which play a major role in controlling antioxidants. It is concluded that a aerial parts of ethyl acetate extract of Chomelia asiatica, which contains large amounts of phenolic compounds, exhibits high antioxidant and free radical scavenging activities. These in vitro assays indicate that this plant extracts is a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses.

Key words: Aerial parts of *Chomelia asiatica*, DPPH assay, *In vitro* antioxidant, Iron chelating activity, Superoxide anion.

INTRODUCTION

Antioxidants are often used in oils and fatty foods to retard their autoxidation. Synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), have restricted use in foods as they are suspected to be carcinogenic. Therefore, the importance of search for natural antioxidants has greatly increased in the recent years[1]. Ethnomedical literature contains a large number of plants that can be used against diseases, in which reactive oxygen species and free radical play important role. There is a plethora of plants that have been found to possess strong antioxidant activity [2]. Recent reports indicate that there is an inverse relationship between the dietary intake of antioxidant-rich foods and the incidence of human diseases[3]. So, many researchers have focused on natural antioxidants and in the plant kingdom numerous crude extracts and pure natural compounds were previously reported to have antioxidant properties. *Chomelia asiatica* (Linn) is belongs to the family Rubiaceae, commonly known as *Tharani in tami*: *Kuppipoovu*, *Tharana* in makayalam[4]. Leaves simple, opposite, decussate; stipules triangular with apiculate tip, interpetiolar, caducous and leaving scar; petiole 0.5-2 cm, canaliculate, glabrous; lamina 8-18 x 4-8 cm, elliptic to elliptic-ovate, apex shortly and abruptly acuminate with blunt tip, base

attenuate, margin entire and revolute, glabrous beneath; midrib usually raised above and canaliculate when dry; secondary_nerves 9-12 pairs, hairy domatia present at axils of secondary_nerves; tertiary_nerves broadly reticulate. The flower Inflorescence terminal corymbose cymes; flowers cream to yellow, subsessile. The fruits are Berry, 2-celled; seeds many[5].

Chomelia Gaertn is a genus of about 370 species distributed in tropical and subtropical Africa, asia,Madagascar and pacific islands. *Chomelia asiatica* (Linn) is a common species which occurs in india ,srilanka and china. The leaves or powder extracts of *Chomelia asiatica* are used as an antimicrobial activities [6](Jayasinghe et al., 2002;). It had been reported analgesic and anti-inflammatory activities[7](Amutha et al., 2012). The parts of *Tarenna asiatica* (Rubiaceae) plants are traditionally used to promote suppuration [8](Anonymous, 1976), as anthelmintic [9](Ramarao and Henry, 1996) and antiulcer agent[10] (Rao et al., 2006). The phytochemical constituents of it are reported to be antiseptic [11](Vinoth-kumar et al., 2011), wound healing[12] (Anjanadevi and Menaga, 2013) and antioxidant[13] (Ramabarathi et al., 2014). Besides, the extract of shoots, leaves and fruits are purportedly active against Mycobacter phlei [14](Rajakaruna et al., 2002).

However, no data are available in the literature on the antioxidant activity of aerial parts of *Chomelia asiatica* (Linn). Therefore we undertook the present investigation to examine the antioxidant activities of various extract of aerial parts of *Chomelia asiatica* (Linn) through various *in vitro* models.

MATERIALS AND METHODS

Collection and identification of plant materials

The aerial parts of *Chomelia asiatica* (Linn), were collected form Senkottai, Tirunelveli District of Tamil Nadu, India. Taxonomic identification was made from Botanical Survey of Medical Plants Unit Siddha, Government of India. Palayamkottai. The aerial parts of *Chomelia asiatica*(Linn), were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve.

Preparation of extracts

The above powered materials were successively extracted with Petroleum ether (40-60^{0}C) by hot continuous percolation method in Soxhlet apparatus[15] for 24 hrs. Then the marc was subjected to Ethyl acetate (76-78^{0}C) for 24 hrs and then mark was subjected to Methanol for 24 hrs. The extracts were concentrated by using a rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained.

Evaluation of antioxidant activity by *in vitro* techniques:

DPPH photometric assay

The effect of extract on DPPH radical was assayed using the method of Mensor et al (2001)[16]. A methanolic solution of 0.5ml of DPPH (0.4mM) was added to 1 ml of the different concentrations of plant extract and allowed to react at room temperature for 30 minutes. Methanol served as the blank and DPPH in methanol without the extracts served as the positive control. After 30 min, the absorbance was measured at 518 nm and converted into percentage radical scavenging activity as follows.

$$Scavengingactivity(\%) = \frac{A_{518}\ \text{Control} - A_{518}\ \text{Sample}}{A_{518}\ \text{Control}} \times 100$$

Where A_{518} control is the absorbance of DPPH radical+ methanol; A_{518} sample is the absorbance of DPPH radical+ sample extract/ standard.

Superoxide radical scavenging activity

Superoxide radical (O_2^-) was generated from the photoreduction of riboflavin and was deducted by nitro blue tetrazolium dye (NBT) reduction method. Measurement of superoxide anion scavenging activity was performed based on the method described by Winterbourne et al (1975)[17]. The assay mixture contained sample with 0.1ml of Nitro blue tetrazolium (1.5 mM NBT) solution, 0.2 ml of EDTA (0.1M EDTA), 0.05 ml riboflavin (0.12 mM) and 2.55 ml of phosphate buffer (0.067 M phosphate buffer). The control tubes were also set up where in DMSO was added instead of sample. The reaction mixture was illuminated for 30 min and the absorbance at 560 nm was measured against the control samples. Ascorbate was used as the reference compound. All the tests were performed

in triplicate and the results averaged. The percentage inhibition was calculated by comparing the results of control and test samples.

Total phenol

The measurement of total phenol is based on Mallick and Singh (1980)[18]. To 0.25g of sample, added 2.5 ml of ethanol and centrifuged at 2^{o}C for 10 mins. The supernatant was preserved. Then, the sample was re-extracted with 2.5 ml of 80% ethanol and centrifuged. The pooled supernatant was evaporated to dryness. Then, added 3 ml of water to the dried supernatant. To which added 0.5 ml of Folins phenol reagent and 2 ml of sodium carbonate (20%). The reaction mixture was kept in boiling water bath for 1 min. the absorbance was measured at 650 nm in a spectrophotometer.

RESULTS AND DISCUSSION

DPPH scavenging activity

DPPH is a stable free radical at room temperature often used to evaluate the antioxidant activity of several natural compounds. The reduction capacity of DPPH radicals was determined by the decrease in its absorbance at 517 nm, which is induced by antioxidants.

The percentage of DPPH radical scavenging activity of petroleum ether extract of *Chomelia asiatica* presented as shown in the Table 1. The petroleum ether extract of *Chomelia asiatica* exhibited a maximum DPPH scavenging activity of 49.72% at 1000 µg/ml whereas for Rutin (standard) was found to be 69.83% at 1000 µg/ml. The IC_{50} of the petroleum ether extract of *Chomelia asiatica* and Rutin were found to be 1040µg/ml and 480µg/ml respectively.

Table 1 Effect of petroleum ether extract of *Chomelia asiatica*(Linn) on DPPH assay

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Petroleum ether extract)	Standard (Rutin)
1	125	12.90±0.014	18.85 ± 0.076
2	250	14.15±0.054	22.08 ± 0.054
3	500	21.10±0.042	52.21 ± 0.022
4	1000	49.72±0.024	69.83 ± 0.014
		IC_{50} = 1040 µg/ml	**IC_{50} = 480 µg/ml**

**All values are expressed as mean ±SEM for three determinations*

As shown in the Table 2, The percentage of DPPH radical scavenging activity of ethyl acetate extract of *Chomelia asiatica* presented in Table 2. The ethyl acetate extract of *Chomelia asiatica* exhibited a maximum DPPH scavenging activity of 65.36% at 1000 µg/ml whereas for Rutin (standard) was found to be 69.83% at 1000 µg/ml. The IC_{50} of the ethyl acetate extract of *Chomelia asiatica* and Rutin were found to be 460µg/ml and 480µg/ml respectively

Table 2 Effect of ethyl acetate extract of *Chomelia asiatica*(Linn) on DPPH assay

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Ethyl acetate extract)	Standard (Rutin)
1	125	35.23 ± 0.042	18.85 ± 0.076
2	250	46.98 ± 0.068	22.08 ± 0.054
3	500	51.52 ± 0.032	52.21 ± 0.022
4	1000	65.36 ± 0.072	69.83 ± 0.014
		IC_{50} = 460 µg/ml	**IC_{50} = 480 µg/ml**

**All values are expressed as mean ±SEM for three determinations*

As shown in the Table 3, The percentage of DPPH radical scavenging activity of methanolic extract of *Chomelia asiatica* exhibited a maximum DPPH scavenging activity of 56.85% at 1000 µg/ml whereas for Rutin(standard) was found to be 69.83% at 1000 µg/ml. The IC_{50} of the methanolic extract of *Chomelia asiatica* and Rutin were found to be 810µg/ml and 480µg/ml respectively.

Table 3 Effect of methanolic extract of *Chomelia asiatica*(Linn) on DPPH assay

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Methanolic extract)	Standard (Rutin)
1	125	12.12±0.042	18.85 ± 0.076
2	250	19.58±0.049	22.08 ± 0.054
3	500	34.16±0.026	52.21 ± 0.022
4	1000	56.45±0.048	69.83 ± 0.014
		IC_{50} = 810 µg/ml	**IC_{50} = 480 µg/ml**

**All values are expressed as mean ±SEM for three determinations*

The ethyl acetate extract of *Chomelia asiatica* was found to more effective than petroleum ether and methanolic extract. The IC_{50} of the ethyl acetate extract of *Chomelia asiatica* and Rutin were found to be 460µg/ml and 480µg/ml respectively.

As shown in the Table 4, the superoxide is a highly reactive molecule that reacts with various substances produced through metabolic processes. Percentage scavenging of superoxide anion examined at different concentrations of petroleum ether extract of *Chomelia asiatica* (125, 250, 500, 1000 µg/ml). The maximum scavenging activity of plant extract and Quercetin at 1000 µg/ml was found to be 66.52% and 98.01% respectively. The IC_{50} value of plant extract and Quercetin was recorded as 460µg/ml and 60µg/ml respectively.

Table 4 Effect of petroleum ether extract of *Chomelia asiatica* (Linn) on superoxide anion scavenging activity method

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Petroleum ether extract)	Standard (Quercetin)
1	125	26.62 ±0 .025	73.81 ± 0.006
2	250	38.45 ± 0.042	91.31 ± 0.011
3	500	54.36 ± 0.034	94.99 ± 0.024
4	1000	66.52 ± 0.026	98.01 ± 0.012
		IC_{50} = 460 µg/ml	**IC_{50} = 60 µg/ml**

**All values are expressed as mean ±SEM for three determinations*

As shown in the Table 5, the percentage scavenging of superoxide anion examined at different concentrations of ethyl acetate extract of *Chomelia asiatica* (125, 250, 500, 1000 µg/ml). The maximum scavenging activity of plant extract and quercetin at 1000 µg/ml was found to be 87.54% and 98.01% respectively.. The IC_{50} value of plant extract and quercetin was recorded as 95µg/ml and 60µg/ml respectively.

Table 5 Effect of ethyl acetate extract of *Chomelia asiatica*(Linn) on superoxide anion scavenging activity method

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Ethyl acetate extract)	Standard (Quercetin)
1	125	53.60 ± 0.034	73.81 ± 0.006
2	250	67.56 ± 0.036	91.31 ± 0.011
3	500	78.40 ± 0.042	92.99 ± 0.024
4	1000	87.54 ± 0.018	98.01 ± 0.012
		IC_{50} = 95 µg/ml	**IC_{50} = 60 µg/ml**

**All values are expressed as mean ±SEM for three determinations*

Table 6 Effect of methanolic extract of *Chomelia asiatica*(Linn) on superoxide anion scavenging activity method

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Methanolic extract)	Standard (Quercetin)
1	125	23.52 ± 0.010	73.81 ± 0.006
2	250	47.29 ± 0.029	91.31 ± 0.011
3	500	73.40 ± 0.035	92.99 ± 0.024
4	1000	89.60± 0.042	98.01 ± 0.012
		IC_{50} = 290 µg/ml	**IC_{50} = 60 µg/ml**

**All values are expressed as mean ±SEM for three determinations*

As shown in the Table 6, the percentage scavenging of superoxide anion examined at different concentrations of methanolic extract of *Chomelia asiatica* (125, 250, 500, 1000 µg/ml) concentration of plant extract. The maximum scavenging activity of plant extract and quercetin at 1000 µg/ml was found to be 89.60% and 98.01% respectively. The IC_{50} value of plant extract and quercetin was recorded as 290µg/ml and 60µg/ml respectively.

Based on the above results the IC_{50} values and percentage scavenging capacity, it was found that ethyl acetate extract of *Chomelia asiatica*is more effective in scavenging superoxide radical than that of methanol and petroleum ether extract. But when compare to the all the three extracts with Quercetin (standard), the ethyl acetate extract of the *Chomelia asiatica* showed the similar result.

As shown in the Table 7, the phenolic compounds are known as powerful chain breaking antioxidants[29]. Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups. The total amount of phenolic content of various extract of aerial parts of *Chomelia asiatica* was present below.

Table 7 The total Phenolic content of various extracts of aerial parts of *Chomelia asiatica*

S.No	Extracts	Total phenol content (mg/g of Catechol) (±SEM)*
1	Petroleum ether extract of *Chomelia asiatica*	2.06 ± 0.032
2	Ethyl acetate extract of *Chomelia asiatica*	8.68 ± 0.046
3	Methanolic extract of *Chomelia asiatica*	6.76 ± 0.054

**All values are expressed as mean ±SEM for three determinations*

Based on the result the methanolic and ethyl acetate extract of *Chomelia asiatica* was found higher content of phenolic components than that of petroleum ether extract of *Chomelia asiatica*.

In conclusion, the results obtained in the present study, the aerial parts of ethyl acetate of *Chomelia asiatica*, which contains large amounts of phenolic compounds, exhibits high antioxidant and free radical scavenging activities. These *in vitro* assays indicate that this plant extracts is a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses. Therefore, further investigations need to be carried out to isolate and identify the antioxidant compounds present in the plant extract.

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